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EFFECTS OF STRAIN AND SEX ON GROWTH PERFORMANCE WITH PROLACTIN GENE EXPRESSION IN DIFFERENT TISSUES OF EXOTIC (COBB 500) AND NIGERIAN IMPROVED CHICKEN STRAINS (NOILER)

[1] Owolabi A. O., [2] Osowe C.O., [3] Atansuyi A.J., [4] Adu O. A., [5] Chineke C. A., [6] Adebayo I.A

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Abstract: **The poultry industry plays a vital role in meeting the growing global demand for animal protein. Genetic improvement of chicken strains is crucial to enhance production efficiency.**

This study investigates the genetic variations in Prolactin (PRL) and its potential associations with growth traits in two distinct chicken strains, Broilers (Cobb 500) and Noilers.

To achieve this, a total of 200 day-old chicks comprising 100 Cobb 500 and 100 Noiler strain of mixed sex were used for the experiment. The study was carried out for a period of eight weeks with the birds distributed into pens based on strain and sexes into twelve treatments (17 birds per pen), genomic DNA samples were collected from the hypothalamus, breast, liver and spleen of both broiler and noiler chickens. Polymerase Chain Reaction (PCR) amplification and sequencing techniques were employed to analyse the PRL genes in the different tissues. The result revealed a significant effect ($p<0.05$) in all tissues except the liver. Notably, higher PRL values were recorded for female Cobb 500 in the liver and hypothalamus and female Noiler in the breast. These findings underscore the strain-specific regulation of PRL expression and highlight distinct gender-associated patterns in different tissues.

Subsequently, growth performance traits, including body weight, feed conversion, and linear measurements were measured in the two strains. Results indicated superior growth performance in Cobb 500 compared to Noiler chickens, with higher final weights observed in the former.

This study sheds light on the genetic underpinnings of growth traits in broiler and noiler chickens, offering valuable insights into potential avenues for selective breeding programs aimed at optimizing poultry production. Understanding the genetic basis of growth traits is pivotal for developing sustainable and economically viable chicken strains, ultimately contributing to the global food security challenge.

Keywords: **Prolactin; Growth traits; Deoxyribonucleic acid; Polymerase chain reaction; Body weight; Feed conversion ratio.**

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1. INTRODUCTION

The contribution of poultry to animal protein supply cannot be over- emphasized (Ahmed *et al*., 2018). Poultry products such as meat and eggs are excellent sources of animal proteins necessary to meet human protein requirements (Olawumi *et al*., 2012). Growth is a complex trait that is controlled by genetic and non- genetic factors (Udeh and Ogbu, 2011). The growth performance is an important and a critical factor to be considered in poultry production as it directly affect the profitability of the industry. According to Yakubu and Salako (2009), growth is a dynamic physiological process that exists from conception until maturity. Animal growth refers to an increase in body size and accumulation of adipose tissues during development from conception to maturity (Ajayi and Ejiofor, 2009). According to Ojedapo *et al* (2016), there are several factors which affect the growth performance of chickens and these includes strain, sex, nutrition, housing and stocking density. Taha *et al*. (2010) reported the significant effect of chicken strain on growth rate and body weight at different ages an body weight and linear body parameters of broilers are dependent on their genotypes (Atansuyi *et al*., 2017). The growth rate of commercial broiler chickens is fast and they are able to reach market weight of two kilogram and above at about seven weeks of age or less (Tallentire *et al*., 2016).

Noiler chicken on the other hand have some qualities that distinguished them from the exotic chicken; they can adapt to harsh weather, disease resistant, hardy and self-reliant. These birds can hatch their eggs, brood their chicks and roam about to feed themselves (Assan, 2015) study had shown that these birds have the ability to produce egg and meat. There were reports on characterization of Nigerian indigenous chickens (Adebambo, 2005) but these birds have not been classified into strains on the basis of their feather colours and shadings. Growth traits are essential parameters in assessing the potentials of genetic improvement and development of any livestock breed/strain. Studies have shown that growth traits measurements such as body length, shank length and chest girth serve as good indicators of growth (Ige, 2013). Yunusa and Adeoti (2014) had earlier reported that skeletal growth and muscular development are interconnected. Chineke (2005), in his study opined that growth traits measurement is useful to study interactions between heredity and environment.

Lastly, genetic factors play a significant role in determining the growth performance of chickens and identifying the genes that are associated with these traits could provide valuable insights into the underlying mechanisms that govern growth performance. Prolactin (PRL) is an important hormones that regulates various physiological processes in birds. Prolactin is produced by the pituitary gland and regulates a wide range of physiological processes, including reproduction, parental behaviour, feather development, and osmoregulation. Prolactin stimulates the production of crop milk in pigeons and doves, which is used to feed their young. There is need to investigate the genetic association of prolactin with growth performance traits and improve on the productivity of our local chicken genetic resource (Ogbu, 2010).

2. MATERIALS AND METHODS

Experimental birds and their population

Two hundred (200) day-old chicks comprising 100 Cobb 500 and 100 Noiler strain of mixed sex were used for the experiment. The study was carried out for a period of eight weeks with the birds distributed into pens based on strain and sexes into twelve treatments.

Experimental Diet

Conventional broiler starter diet containing 2800Kcal/kg metabolizable energy and 22% crude protein was used for the experiment for the first four weeks while broiler finisher diet containing 2900Kcal/kg metabolizable energy and 20% crude protein was fed to the birds for the last four weeks.

Management of experimental birds

The pens were swept, thoroughly washed and disinfected prior the arrival of the chicks. Also, the feeders and drinkers were washed, sanitized and dried. The initial weight of the chicks were taken on arrival and they were tagged individually for ease of weighing, measurement and recording. The chicks were distributed into the pens according to their strain and sexes and were raised intensively for a period of eight weeks**.** Routine and occasional health management practices were strictly observed during the period of the study. The birds were vaccinated and medicated accordingly.

Growth performance, morphometric measurements and Data collection

The initial weight of all chicks were taken at day old using sensitive scale (5kg *Max*) and the chicks were tagged individually for ease of identification, measurement and weighing. Subsequently, their body weights were taken weekly for eight weeks. The body measurements were taken individually before feeding in the morning after which the mean body weight for all the birds were recorded based on their sexes and strains. Growth parameters were measured through feed consumed, feed conversion ratio and weight gain for each strain and sex. The measurements taken were:

Body Weight gain (BWG): This is the difference between the final live weight and the initial live weight measured in grams using a sensitive scale (Weight gain= Final body weight-Initial body weight)

Feed intake (FI): This was determined by measuring the difference between feed supplied and remnants on weekly basis.

Feed intake (FI) = Feed consumed – Feed remnants (weigh back)

Feed conversion ratio (FCR): This was obtained by dividing the weight gain (WG) by feed intake (FI). (FCR = FI ÷ WG)

The morphometric traits measured includes Body weight (BWT), Breast girth (BRG), Neck circumference (NC), Drumstick (DST), Shank length (SHL), Shank circumference (SC), Wing length (WGL), Body circumference (BC), Shoulder to tail length (STL), Beak length (BKL).

The description of the linear body measurements studied were given below:

Body weight (BWT): Body weight of each bird was measured in grams with a sensitive scale (*5kg Max*)

Breast girth (BRG): This is measured as the body circumference under the wings

Neck circumference (NC): This refers to the measurement round or circumference of the neck

Drumstick (DST): This refers to the measurement of the hinge and the hock joints

Shank length (SHL): This refers to the distance between the hock joint and the extremities of the *digitus pedis***.**

Shank circumference (SC): This is the measurement round the lower led or shank of the chicken

Wing length (WGL): It was measured by stretching the wing and the measurement taken from humerus junctions to the tip of the wing.

Body circumference (BC): Refers to the measurement round the body under the wings

Shoulder to tail length (STL): This is measured from the wing to the tip of the tail

Beak length (BKL): This is measured from the base of the nostrils to the tip of the beak

Gene Expression Analysis

Tissue materials and Sample collection

Tissues from the Hypothalamus, liver, spleen and breast were collected from each of the selected birds of mixed sex. These tissue samples were harvested into well labelled test tubes containing RNA later and were stored immediately at a temperature of 4^{o}C and -80 ^{o}C respectively to preserve the RNA prior further laboratory analysis.

RNA Extraction

Total RNA was extracted from the selected samples using FIREScript RT cDNA synthesis KIT. Tissues from the hypothalamus, liver, breast and spleen were collected from each of the selected birds of mixed sex into specimen bottles containing RNA Later. Sample preparation was performed at room temperature by homogenizing each sample in 600µl RNA Lysis Buffer followed by purification of the total RNA after which the quality and quantity was assessed using nanodrop 2000c spectrophotometer.

Synthesis of cDNA

After the RNA extraction, Complementary DNA (cDNA) was synthesized from 1 µg of RNA using FIREScript Reverse transcriptase (RT) kit in a 3-step reaction at 25^{o}C for 5minutes, 60^{o}C for 30 minutes and 85^{o}C for 5 minutes respectively after which forward and reverse primer sequence was designed while Beta actin gene was obtained from Genbank.

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Table 1: Oligonucleotide primer sequences used for PRL gene expression

Gene	Sequence	TS	Tm (^{0}C)	GC (%)
PRL	F: GCTGCCACACTTCCTCCTTA	Plus	55.00	19.00
	R: GGCGGGTTCATTCTGGTCAT	Minus	55.00	22.00
β-Actin	F: GCCTGGAGTGTATAAAGCACACA	Plus	60.89	55.00
	R: GCTGACAAGAGCATGAGTCC	Minus	59.96	55.00

TS = Template Strand, Tm = Melting Temperature, GC = Guanine-Cytosine content, PRL = Prolactin.

Real-time PCR

Quantitative Polymerase Chain Reaction (qPCR) was performed on all the samples with HOT FIREPol EvaGreen qPCR master mix. The gene was executed using Applied Biosystems Step-One Plus real time PCR

Analytical procedures

qPCR assays were prepared and run in Roche LightcyclerR 96 USA. The quantification cycle (cq) means were used as the basis for analysis of the gene expression. Expression of the gene (PRL) was stabilized using the reference gene (β-Actin). Gene expression was calculated using the formula Cq=mean Cq (target gene)-mean Cq (reference gene) (Busting *et al*., 2009). A high Cq value indicates high transcriptase.

Statistical Analysis

Data obtained were subjected to analysis of variance (SAS, 2018) and means were separated using Multiple Range Test. The experimental design used was 2× 2 factorial arrangement in a Completely Randomised Design. The Statistical model is stated below

$Y_{ijk} = \mu + B_i + S_j + G_k + BS_{ij} + BG_{ik} + SG_{jk} + BSG_{ijk} + \varepsilon_{ijk}$

Where Y_{ijk} = individual observation

μ = overall means

B_i = fixed effect of *i* strain (I = 1, 2)

S_j = fixed effect of *j* sex j = (1, 2)

G_k = random effect of gene (1, 2)

BS_{ij} = interaction between strain & sex

BG_{ik} = interaction between strain & gene

SG_{jk} = interaction between sex and gene

BSG_{ijk} = interaction between strain, sex and gene

ε_{ijk} = residual error

3. RESULTS AND DISCUSSION

Effects of strain and sex on the growth performance of the experimental birds

Table 1 shows the growth performance by strain and sex of the two experimental birds. There were significant differences ($p<0.05$) in the IWT, FWT, TWG and FCR of the two strains. It was observed that Cobb 500 weighed heavier (6796.92±608.58g) than Noiler strains (3930.06±608.58g). FWG was also higher in Cobb 500 (9181.60±718.48g) than Noiler strains (5097.00±718.48g). It was also observed that Cobb 500 have FCR of 2.84g as against that of Noiler strains which recorded (4.93g). TWG was higher in Cobb500 (1169.02g) than in Noiler (2400.48g). There was no significant difference ($p>0.05$) in TFI of both Cobb 500 and Noiler strains.

Sex did not have significant ($p>0.05$) effect on the growth performance of the two strains of experimental birds (Cobb 500 and Noiler)

The interaction between strain and sex did not show significant differences. ($p>0.05$)

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Table 1. Effects of strain and sex on the growth performance of the experimental birds

Factor	Parameter	IWT	TFI	TWG	FCR	FWT
Breed	Cobb500	6796.92[a]	5826.10	2400.48[a]	2.84[a]	9181.60[a]
	Noiler	3930.06[b]	5758.48	1169.02[b]	4.93[b]	5097.00[b]
	±SEM	608.58	345.00	149.77	0. 20	718.48
	P-value	0.001	0.890	0.001	0.001	0.001
Sex	Male	5466.40	5952.90	1691.65	3.96	7155.96
	Female	5260.58	5631.69	1877.85	3.81	7122.65
	±SEM	608.58	345.00	149.77	0.20	718.48
	P-value	0.81	0.51	0.38	0.59	0.97
Breed*Sex						
Cobb500	Male	6841.79	6115.25	2166.58	3.04	9008.38
	Female	6752.04	5536.96	2634.38	2.64	9354.83
Noiler	Male	4091.00	5790.54	1216.71	4.89	5303.54
	Female	3769.13	5726.42	1121.33	4.98	4890.46
	±SEM	860.67	487.91	211.81	0.29	1016.08
	P-value	0.89	0.60	0.19	0.38	0.71

a.b.c Means in the same column bearing different superscripts are significantly different ($p<0.05$). IWT = Initial weight, FWT = Final weight, TFI = Total feed intake, TWG = Total weight gain and FCR = Feed conversion ratio

Expression of PRL on the selected tissues of the experimental birds by strain and sex

In **Table 2** Effects of PRL by the strain of chicken were significantly expressed ($p<0.05$) in all the tissues (hypothalamus, breast, liver and spleen) except in the liver which was not significant ($p>0.05$). PRL values were higher in all the tissues in Cobb 500 compared with noiler. Effects of sex on the expression of the hormonal marker PRL was significant on breast. However, there were no significant effects ($p>0.05$) of PRL on hypothalamus, liver and spleen. PRL was higher (1.18) in the breast of female, liver of male (1.19) and spleen of male (0.72).

The interaction between strain and sex were not significant ($p>0.05$) on the expression of PRL in the hypothalamus, liver and spleen while significant effect ($p<0.05$) was expressed by PRL in the breast.

Table 2. Expression of PRL on the selected tissues of the experimental birds by strain and sex

		HYPO	**BREAST**	**LIVER**	**SPLEEN**
Factor	**Sex**	**PRL**	**PRL**	**PRL**	**PRL**
Breed	Cobb	1.16[a]	0.98[a]	1.21	0.93[a]
	Noiler	0.10[b]	0.69[b]	1.06	0.44[b]
	±SEM	0.66	0.02	0.07	0.02
	P-Value	0.00	0.00	0.17	0.00
Sex	Male	0.61	0.49[b]	1.19	0.72
	Female	0.61	1.18[a]	1.07	0.64
	±SEM	0.66	0.02	0.07	0.02
	P-Value	0.90	0.00	0.25	0.07
Breed*Sex					
COBB	Male	1.10[a]	0.88[c]	1.12	0.93[a]
	Female	1.13[a]	1.07[b]	1.16	0.92[a]
Noiler	Male	0.11[b]	0.09[d]	1.14	0.51[b]
	Female	0.10[b]	1.29[a]	0.98	0.36[c]
	±SEM	0.15	0.13	0.05	0.07
	P-Value	0.00	0.00	0.33	0.00

a.b.c means in the same column bearing different superscripts are significantly different ($p<0.05$).HYPO = Hypothalamus, PRL= Prolactin.

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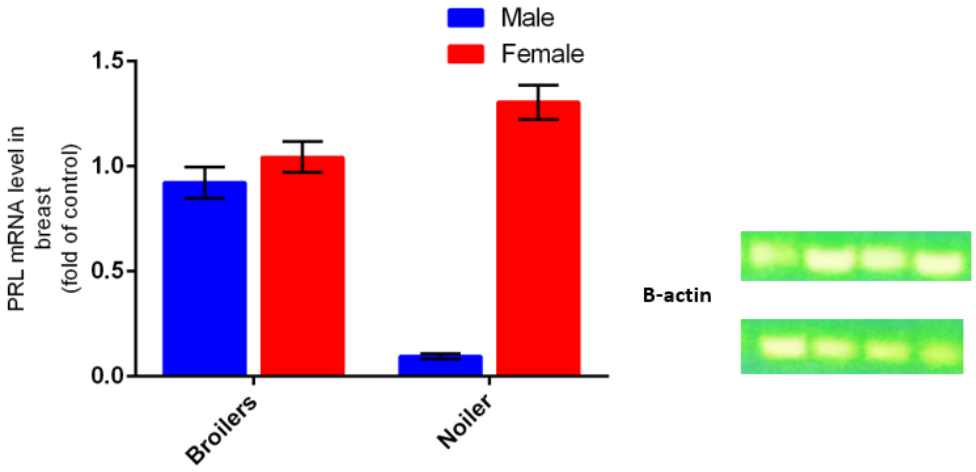

Figure 1: Gene expression level of Prolactin in the breast of the two strains of chicken using real time qPCR.

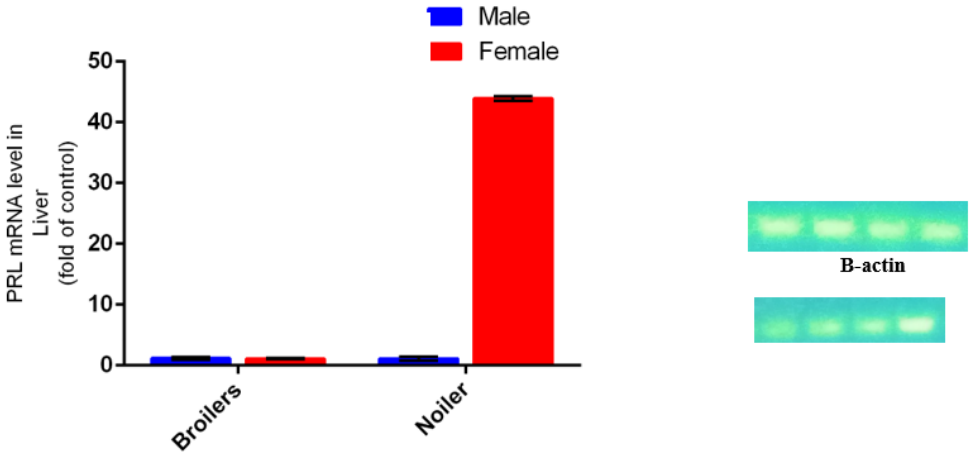

Figure 2: Gene expression level of Prolactin in the Liver of the two strains of the experimental birds using real time qPCR.

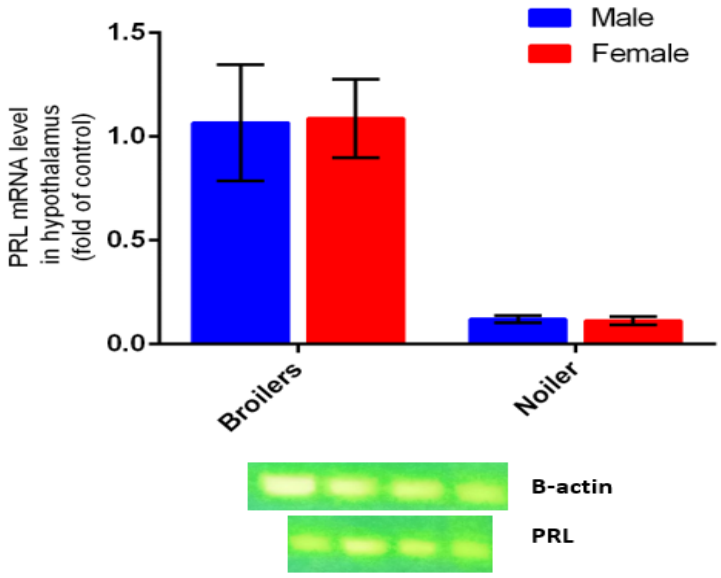

Figure 3: Gene expression level of Prolactin in the hypothalamus of Cobb 500 and Noiler chicken strains using real time qPCR.

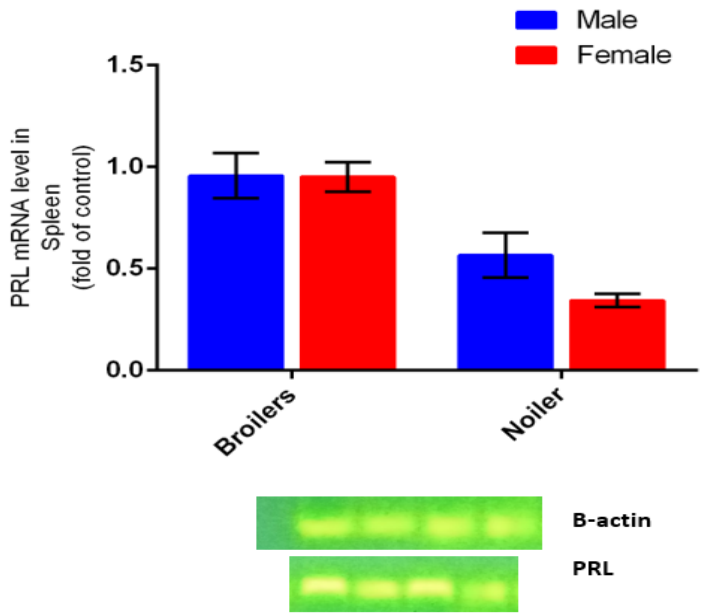

Figure 4: Gene expression level of Prolactin in the Spleen of Cobb 500 and Noilers (male and female) using real time qPCR.

Figure 1 shows the expression level of Prolactin in the breast of the two strains of the experimental birds using real time qPCR. The pictogram shows that PRL was higher in female noiler than female broilers, so also higher in male broilers than male noilers.

Figure 2 shows the expression level of Prolactin in the Liver of the two strains of the experimental birds using real time qPCR. It was reflected in the figure that prolactin was highly expressed in the liver of the female noiler chicken as against the broilers (male and female) as well as the male noilers.

The expression level of Prolactin in the hypothalamus of Cobb 500 and Noiler chicken strains is presented in **Figure 3** Prolactin was highly expressed in broilers as against noiler chicken strains. The expression was higher in female broilers than the male while it was higher in male noilers than the female noilers

It was shown in **Figure 4** that prolactin was highly expressed in the spleen of Cobb 500 compared with Noilers. The expression was higher in male broilers than female broilers and higher in male noilers than the female noilers.

4. DISCUSSION

Associations between PRL with growth performance in Broilers and Noilers

The result of the growth performance of the experimental birds showed that Cobb 500 performed better than Noiler chickens. The final weight of Cobb 500 was higher than the final weight of Noiler chickens. This agreed with Tallentire *et al* (2016), who reported that broilers are characterized with higher body weights compared with other indigenous chickens. According to Fasina and McCrindle (2003), the reason for lower growth performance in Noiler might be as a result of feed quality, management practices, genetic variations and environmental conditions.

It was also observed from the result that Cobb 500 had higher FCR than Noiler, this implied that Cobb 500 had a higher feed conversion ratio which resulted into higher weight gain and consequently, higher final weight. This agreed with Bessei (2006) who stated that "broilers are characterized with high feed conversion. Feed conversion ratio is an indirect indicator of performance efficiency. It indicates the amount of feed consumed to obtain one unit of weight gain on the live bird in a given period of time. It is expressed as the ratio of feed intake over live weight gain. (Emmerson, 1997).

Sex did not have significant effect on the growth performance of the two strains of experimental birds and this could be attributed to similarities in the genetic factors.

The result showed significant effect of strain on body weight and neck circumference. Higher body weight was recorded in Cobb 500 as against Noiler strain.

Several genetic variants within these genes have been associated with growth-related traits in chickens. Sheng *et al*. (2019) reported significant associations between certain genetic variants and body weight at different growth stages. Prolactin (PRL) have been identified as potential candidate gene influencing growth-related traits.

The result obtained from **Table 2** showed that there were significant effects of strain on the expression of PRL in all the tissues except liver where there was no significant effect and higher values were also recorded for female Cobb 500 in the hypothalamus, female noiler in the breast and female Cobb in the liver. This agreed with Li *et al* (2020) who stated that prolactin is a female hormone associated with broodiness and parental behavior in chickens. Based on the current findings, prolactin influences the hypothalamus by affecting the secretion of gonadotrophin-releasing hormone (GnRH). In broilers and noilers, variations in prolactin expression could impact the reproductive and growth-related functions regulated by GnRH. However, elevated prolactin levels have been linked to reduced growth rates in broilers and subsequently with the suppression of GnRH secretion thereby affecting reproductive performance (Li *et al*., 2020).The differences in PRL levels among strains may result into susceptibility to diseases among the strains (Mo *et al*., 2022) Prolactin plays essential roles in metabolism regulation of the immune system and pancreatic development. (Lucas *et al*., 1998). Elevated PRL level decreases the level of sex hormones (estrogen in female, testosterone in male). According to the findings of this current study, PRL is associated with onset of broodiness in chickens and also triggers the hens desire to sit on her eggs for incubation. High level of PRL suppresses egg-laying during broodiness conversely, prolactin may stimulate egg production during non-brooding period. PRL can also influence feather growth. Elevated PRL due to broodiness may bring about changes in feather cover as they pluck feather for nest lining and also enhance maternal and nurturing instinct. PRL is not only a pituitary hormone that plays a major role in reproduction, but also acts as a cytokine in the immune response. PRL can influence the local environment of immune organs and contribute to the maturation and function of immune cells. The presence of PRL significantly improves the proliferation of immune cells (Fojtíková *et al.*, 2010).PRL inhibits lymphocyte proliferation at high concentrations and enhances proliferation at low concentrations (Nela *et al*., 2014; Suarez *et al*., 2015). The high expression of PRL in the female Cobb and noiler may enhance the immune response of the birds, but the continuous high expression may lead to the occurrence of autoimmune diseases. PRL itself cannot initiate the immune response, and mainly maintains the balance of immune response in the body (Fojtíková *et al*., 2010). Individuals with high PRL levels have relatively strong resistance to pathogens. (Mohammadpour *et al*., 2018; Mo *et al*., 2021). Prolactin can influence body weight and fat deposition as hens divert energy and nutrients to support egg production and incubation. High levels inhibits growth by diverting energy away from muscle development. Effect of sex on the expression of PRL was not significant on hypothalamus, liver and spleen while significant ($p<0.05$) effect was obtained in breast. Some well-established stimulators of pituitary prolactin secretions affect hypothalamus prolactin production i.e ovarian steroids modulates hypothalamus synthesis and release of prolactin (Montgomery *et al*., 1990). Immune-competent cells from thymus and spleen as well as peripheral lymphocytes contain PRL mRNA and release bioactive PRL similar to pituitary prolactin (Devito *et al.*, 1992). Lymphocyte contains dopamine receptors that may be involved in lymphocytic prolactin production. A great deal suggests that lymphocytes can be a source of prolactin (Devins *et al*., 1992).

Interaction showed that PRL in hypothalamus, liver and spleen were significant ($p<0.05$). These results implied that there are variations in the expression of PRL. Furthermore, Li *et al*. (2020) reported that certain genetic variants were associated with body weight, feed conversion rate, and carcass traits. Genetic variations within these genes have been associated with body weight, body length, feed conversion ratio, and carcass traits, indicating their potential as candidate genes for selective breeding programs aimed at improving growth performance in poultry.

Based on the findings of the current study, genes can be up-regulated or down-regulated. When a gene is up-regulated, it means that the expression of that gene is increased, resulting in higher levels of the gene product (e.g. protein). In the case of the prolactin gene, up-regulation might lead to increased production of PRL, a hormone involved in various physiological processes, including reproduction and parental care. Conversely, when a gene is down-regulated, its expression is decreased, leading to lower levels of the gene product. Down-regulation of the prolactin gene could result in reduced prolactin production, which may have implications for reproductive and maternal behaviours in chickens. Gene regulation in various tissues can be influenced by a wide range of factors, including hormones, environmental conditions, genetics, and more. Many genes, including PRL are regulated by hormones. For example, in the hypothalamus and breast tissues, PRL gene expression can be influenced by the hormone prolactin itself. Environmental factors such as temperature, light exposure, and stress can impact gene expression. Stressors like heat stress or exposure to different light regimes can affect the hormonal balance and subsequently the expression of these genes in various tissues. Genetic factors also plays significant role in gene regulation; variations in the genetic makeup of Cobb 500 and Noiler chickens may lead to differences in the

regulation of these genes in different tissues. Dietary factors can also influence gene expression i.e the availability of specific nutrients and their impact on hormonal signaling pathways can affect the expression of genes related to growth, reproduction, and metabolism, which may be relevant to Cobb 500 and Noiler chickens. Infection and diseases can lead to immune responses that impact gene regulation. The spleen, as a part of the immune system, may respond to infections, which can influence gene expression in the tissues of Cobb 500 and Noiler. Lastly, DNA methylation and histone modifications can play a role in gene regulation. These modifications can be influenced by various factors, including diet and environmental conditions.

5. CONCLUSION

- Based on the findings of this study, PRL appeared to have an inhibitory effect on the growth performance in broiler (Cobb 500) and noiler chickens. Elevated PRL levels due to up-regulation have been associated with reduced growth rates. This suggests that managing PRL levels may be a strategy for improving growth performance in both broiler (Cobb 500) and noiler chicken.
- The significant strain-dependent impact on PRL expression across multiple tissues emphasizes the intricate genetic regulation of this hormone in broiler and Noiler chicken strains. The absence of significant effects ($p>0.05$) in the liver suggests a unique regulatory mechanism for PRL in this organ. Furthermore, the observed gender-specific variations in PRL expression highlight the complexity of hormonal dynamics in different tissues, providing valuable insights for understanding the reproductive and physiological differences between Cobb 500 and Noiler chickens.

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